CLAIMS

What is claimed is:

1. An isolated polypeptide comprising in amino to carboxy order P4-P3-P2-P1, wherein P4 is P, P3 is R or K, P2 is any amino acid, and P1 is K or R (SEQ. ID. NO: 1).

2. The isolated polypeptide of claim 1, wherein P4 is amino-terminally blocked.

3. The isolated polypeptide of claim 2, wherein P4 is acetylated.

4. The isolated polypeptide of claim 2, further comprising a fluorogenic leaving group that is covalently bound to P4-P3-P2-P1 at a carboxy-terminus of P4-P3-P2-P1.

5. The isolated polypeptide of claim 4, wherein the fluorogenic leaving group is bound via an amide bond.

6. The isolated polypeptide of claim 4, wherein the fluorogenic leaving group comprises 7-amino-4-carbamoylmethyl-coumarin.

7. The isolated polypeptide of claim 1, wherein P2 is N and further comprising a fluorogenic leaving group that is bound to P4-P3-P2-P1 via an amide bond on a carboxy-terminus of P4-P3-P2-P1

8. The isolated polypeptide of claim 7, wherein the fluorogenic leaving group comprises 7-amino-4-carbamoylmethyl-coumarin.

9. The isolated polypeptide of claim 6, wherein P4-P3-P2-P1 is selected from the group consisting of P-R-N-K (SEQ. ID. NO: 2), P-K-N-K (SEQ. ID. NO: 3), P-R-N-R (SEQ. ID. NO: 4), P-K-N-R (SEQ. ID. NO: 5), P-A-N-K (SEQ. ID. NO: 6), and P-R-T-K (SEQ. ID. NO: 7).

10. The isolated polypeptide of claim 9, wherein P4-P3-P2-P1 is P-R-N-K (SEQ. ID. NO: 2).

11. The isolated polypeptide of claim 9, wherein P4-P3-P2-P1 is P-K-N-K (SEQ. ID. NO: 3).

12. The isolated polypeptide of claim 9, wherein P4-P3-P2-P1 is P-R-N-R (SEQ. ID. NO: 4).

13. The isolated polypeptide of claim 9, wherein P4-P3-P2-P1 is P-K-N-R (SEQ. ID. NO: 5).

14. The isolated polypeptide of claim 9, wherein P4-P3-P2-P1 is P-A-N-K (SEQ. ID. NO: 6).

15. The isolated polypeptide of claim 9, wherein P4-P3-P2-P1 is P-R-T-K (SEQ. ID. NO: 7).

16. The isolated polypeptide of claim 1, wherein P1 is linked to a serine protease reactive inhibitor moiety.

17. The isolated polypeptide of claim 16, wherein the serine protease reactive inhibitor moiety is chloromethyl ketone, which is linked to P1.

18. The isolated polypeptide of claim 16, wherein P4 is amino-terminally blocked.

19. The isolated polypeptide of claim 18, wherein P4 is acetylated.

20. The isolated polypeptide of claim 18, wherein P2 is N.

21. The isolated polypeptide of claim 20, wherein P4-P3-P2-P1 is selected from the group consisting of P-R-N-K (SEQ. ID. NO: 2), P-K-N-K (SEQ. ID. NO: 3), P-R-N-R (SEQ. ID. NO: 4), P-K-N-R (SEQ. ID. NO: 5), P-A-N-K (SEQ. ID. NO: 6), and P-R-T-K (SEQ. ID. NO: 7).

22. The isolated polypeptide of claim 21, wherein P4-P3-P2-P1 is P-R-N-K (SEQ. ID. NO: 2).

23. The isolated polypeptide of claim 21, wherein P4-P3-P2-P1 is P-K-N-K (SEQ. ID. NO: 3).

24. The isolated polypeptide of claim 21, wherein P4-P3-P2-P1 is P-R-N-R (SEQ. ID. NO: 4).

25. The isolated polypeptide of claim 21, wherein P4-P3-P2-P1 is P-K-N-R (SEQ. ID. NO: 5).

26. The isolated polypeptide of claim 21, wherein P4-P3-P2-P1 is P-A-N-K (SEQ. ID. NO: 6).

27. The isolated polypeptide of claim 21, wherein P4-P3-P2-P1 is P-R-T-K (SEQ. ID. NO: 7).

28. A method of assaying activity of an enzymatically-active β-tryptase in a sample, the method comprising:

(a) contacting the sample with an isolated polypeptide comprising in amino to carboxy order P4-P3-P2-P1, wherein P4 is amino-terminally blocked and is P, and wherein P4-P3-P2-P1 is selected from the group consisting of P-R-N-K (SEQ. ID. NO: 2), P-K-N-K (SEQ. ID. NO: 3), P-R-N-R (SEQ. ID. NO: 4), P-K-N-R (SEQ. ID. NO: 5), P-A-N-K (SEQ. ID. NO: 6), and P-R-T-K (SEQ. ID. NO: 7), and further wherein a fluorogenic leaving group comprising 7-amino-4-carbamoylmethyl- coumarin is bound via an amide bond to P4-P3-P2-P1 at a carboxy-terminus of P4-P3-P2-P1, under conditions wherein an amount of the fluorogenic leaving group is cleaved from

P4-P3-P2-P1 upon action of the β-tryptase, thereby producing a fluorescent moiety; and then

(b) quantifying the amount of detectable leaving group cleaved from the polypeptide, the amount being an indication of the activity of the enzymatically-active β-tryptase in the sample.

29. The method of claim 28, wherein in step (a), the detectable leaving group is a fluorogenic leaving group.

30. The method of claim 29, wherein in step (a), the fluorogenic leaving group is attached to a carboxy-terminus of P4-P3-P2-P1 via an amide bond.

31. The method of claim 29, wherein in step (a), P4 is acetylated.

32. The method of claim 31, wherein in step (b), the amount of detectable leaving group cleaved from the polypeptide is detected by observing whether the sample undergoes a detectable change in fluorescence.

33. The method of claim 28, wherein the sample is a bodily fluid clinical sample.

34. The method of claim 33, wherein the clinical sample is whole blood, serum, plasma, urine, tears, lavage, tissue extract, or conditioned media.

35. The method of claim 28, further comprising, prior to step (a), adding aprotinin to the sample to inhibit proteases other than β-tryptase, thereby reducing non-specific cleavage of the detectable leaving group from P4-P3-P2-P1 by proteases other than β-tryptase.

36. A method of assaying activity of an enzymatically-active β-tryptase in a sample, the method comprising:

(a) contacting the sample with an isolated polypeptide comprising in amino to carboxy order P4-P3-P2-P1, wherein P4 is amino-terminally blocked, and wherein P4-P3-P2-P1 is selected from the group consisting of P-R-N-K (SEQ. ID. NO: 2), P-K-N-K (SEQ. ID. NO: 3), P-R-N-R (SEQ. ID. NO: 4), P-K-N-R (SEQ. ID. NO: 5), P-A-N-K (SEQ. ID. NO: 6), and P-R-T-K (SEQ. ID. NO: 7), and further wherein a fluorogenic leaving group comprising 7-amino-4-carbamoylmethyl- coumarin is bound via an amide bond to P4-P3-P2-P1 at a carboxy-terminus of P4-P3-P2-P1, under conditions wherein an amount of the fluorogenic leaving group is cleaved from P4-P3-P2-P1 upon action of the β-tryptase, thereby producing a fluorescent moiety; and then

(b) measuring whether the sample undergoes a detectable change in fluorescence, the detectable change being an indication of the activity of the enzymatically-active β-tryptase in the sample.

37. The method of claim 34, further comprising adding aprotinin to the sample to inhibit proteases other than β-tryptase, thereby reducing non-specific cleaveage of the fluorogenic leaving group from P4-P3-P2-P1 by proteases other than β-tryptase.

38. A method of inhibiting an enzymatically-active β-tryptase in a sample, the method comprising: contacting the sample with an isolated polypeptide comprising in amino to carboxy order P4-P3-P2-P1, wherein P4 is P, P3 is R or K, P2 is any amino acid, and P1 is K or R (SEQ. ID. NO: 1), wherein P4 is acetylated, and wherein P1 is linked to a chloromethyl ketone, under conditions wherein the isolated polypeptide interacts with and inhibits enzymatic β-tryptase present in the sample.

39. The method of claim 38, further comprising quantifying inhibition of the β-tryptase activity in the sample.

40. The method of claim 38, wherein in step (a), P4-P3-P2-P1 is selected from the group consisting of P-R-N-K (SEQ. ID. NO: 2), P-K-N-K (SEQ. ID. NO: 3), P-R-N-R (SEQ. ID. NO: 4), P-K-N-R (SEQ. ID. NO: 5), P-A-N-K (SEQ. ID. NO: 6), and P-R-T-K (SEQ. ID. NO: 7).

41. A kit for analyzing samples for β-tryptase activity comprising:

an isolated polypeptide comprising in amino to carboxy order P4-P3-P2-P1, wherein P4 is P, P3 is R or K, P2 is any amino acid, and P1 is K or R (SEQ. ID. NO: 1), and wherein a detectable leaving group is covalently bound to P4-P3-P2-P1; and

a suitable container, the isolated polypeptide being disposed therein.

42. The kit of claim 41, wherein the isolated polypeptide is provided in solution, lyophilized, or bound to a solid support.

43. The kit of claim 41, wherein P4-P3-P2-P1 further comprises a serine protease reactive moiety.

44. The kit of claim 41, wherein P4 of the isolated polypeptide is acetylated.

45. The kit of claim 41, wherein the detectable leaving group is a fluorogenic leaving group covalently bonded to a carboxy-terminus of P4-P3-P2-P1 via an amide bond.

46. The kit of claim 41, wherein P4-P3-P2-P1 is selected from the group consisting of P-R-N-K (SEQ. ID. NO: 2), P-K-N-K (SEQ. ID. NO: 3), P-R-N-R (SEQ. ID. NO: 4), P-K-N-R (SEQ. ID. NO: 5), P-A-N-K (SEQ. ID. NO: 6), and P-R-T-K (SEQ. ID. NO: 7).

47. The kit of claim 41, further comprising a supply of aprotinin disposed in a second container.

48. The kit of claim 41, wherein P1 is linked to a chloromethyl ketone.